WellReady™

96 Well In-Plate Preservation

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1 Components

1.1 Kit contents

Product Code	Components	Units	Unit Volume	Medium to Add
WR-Z096-001	Gel A (5x)	1 tube	2.2 mL	8.8 mL
	Gel B (5x)	1 tube	2.2 mL	8.8 mL
	Gel C (5x)	1 tube	2.2 mL	8.8 mL
	Gelation Buffer 1	1 tube	10 mL	-
	Gelation Buffer 2	1 tube	10 mL	-
	Dissolution Buffer	2 tubes	20 mL	-
	Adhesive Plate Seals	2	-	-
WR-Z096-003	Gel A (5x)	3 tubes	2.2 mL	8.8 mL
	Gel B (5x)	3 tubes	2.2 mL	8.8 mL
	Gel C (5x)	3 tubes	2.2 mL	8.8 mL
	Gelation Buffer 1	2 tubes	15 mL	-
	Gelation Buffer 2	2 tubes	15 mL	-
	Dissolution Buffer	6 tubes	20 mL	-
	Adhesive Plate Seals	4	-	-
WR-Z096-006	Gel A (5x)	6 tubes	2.2 mL	8.8 mL
	Gel B (5x)	6 tubes	2.2 mL	8.8 mL
	Gel C (5x)	6 tubes	2.2 mL	8.8 mL
	Gelation Buffer 1	3 tubes	20 mL	-
	Gelation Buffer 2	3 tubes	20 mL	-
	Dissolution Buffer	12 tubes	20 mL	-
	Adhesive Plate Seals	7	-	-
WR-Z096-012	Gel A (5x)	12 tubes	2.2 mL	8.8 mL
	Gel B (5x)	12 tubes	2.2 mL	8.8 mL
	Gel C (5x)	12 tubes	2.2 mL	8.8 mL
	Gelation Buffer 1	1 bottle	120 mL	-
	Gelation Buffer 2	1 bottle	120 mL	-
	Dissolution Buffer	24 tubes	20 mL	-
	Adhesive Plate Seals	13	-	-

1.1 Kit contents (continued)

Product Code	Components	Units	Unit Volume	Medium to Add
WR-Z096-024	Gel A (5x)	24 tubes	2.2 mL	8.8 mL
	Gel B (5x)	24 tubes	2.2 mL	8.8 mL
	Gel C (5x)	24 tubes	2.2 mL	8.8 mL
	Gelation Buffer 1	2 bottles	120 mL	-
	Gelation Buffer 2	2 bottles	120 mL	-
	Dissolution Buffer	48 tubes	20 mL	-
	Adhesive Plate Seals	25	-	-

NOTE: Remove components from 2-8°C storage for at least 20 minutes before use

1.2 Components to be supplied by the user

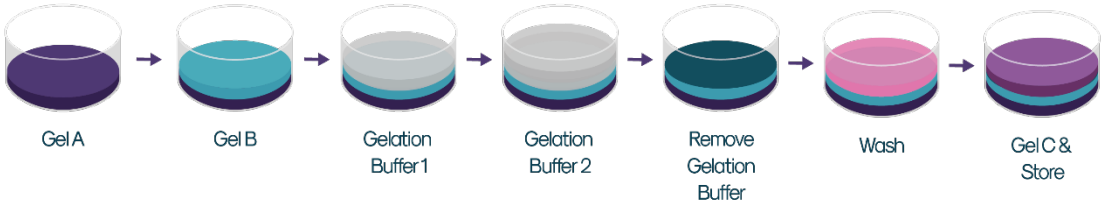
96-well plate with adherent cell cultures

1000 µL and 200 µL micropipettes and tips (multichannel pipette is optional)

Cell Culture Medium

2 Step-by-Step guide

2.1 Overview



2.2 Gelation

1. Ensure that all components are allowed to equilibrate to room temperature before use and conduct all steps in a laminar flow hood at room temperature.
2. Dilute Gel A, Gel B and Gel C 1 in 5 with complete culture medium by adding directly to tubes containing gel concentrate (See kit contents on Page 3 for a dilution guide). Either mix by vortexing for 10 seconds or gently mix with a pipette until homogenous.
3. Carefully remove culture medium from each well of the plate.
4. Add 0.09 mL of the diluted (see step 1) Gel A Solution to each well.
5. Gently add 0.09 mL of the diluted (see step 1) Gel B Solution.
6. Add 0.05 mL of Gelation Buffer 1 (GB1) dropwise over the surface of Gel A/B solution. Allow 10 minutes for Gelation.
7. Add 0.05 mL of Gelation Buffer 2 (GB2) dropwise over the surface of Gel A/B. Allow a further 10 minutes for gelation.
8. Remove GB1/GB2 mixture from each well and wash for 5 minutes with 0.2 mL culture medium per well.

9. Remove culture medium and add 0.09 mL of the diluted (see step 1) Gel C solution to the centre of the gelled surface.
10. Place adhesive plate seal over the surface of the plate ensuring it is properly sealed.
11. Replace the lid and store away from light at an appropriate temperature (either 2-8°C in a refrigerator, or between 10 and 20°C in Controlled Room Temperature (CRT) Packaging or in a temperature-controlled room).[1] Allow to settle for at least 4 hours before shipping.

[1]Atelerix can recommend an appropriate storage temperature for your particular cell type. Please contact technical@atelerix.co.uk

2.3 Release

1. Ensure that all components are allowed to equilibrate to room temperature before use and conduct all steps in a laminar flow hood at room temperature.
2. Remove plate seals and infuse 0.1 mL of Dissolution Buffer onto the gel in each well of the plate. Allow **5 minutes** for gel dissolution. (N.B. to aid dissolution pierce the surface of the gel with the pipette tip during infusion).
3. Carefully remove 0.2 mL of the well contents ensuring that you aspirate from the upper part of the well.
4. Add 0.2 mL Dissolution Buffer and allow a further **5-10 minutes** for full gel dissolution.
5. Remove 0.4 mL (remaining contents of each well) and wash monolayers briefly with 0.1 mL Complete Culture Medium.
6. Add a sufficient volume of Complete Culture Medium and return to normal culture conditions for at least **4 hours** or overnight.
7. Cells are ready for continued culture or for downstream analysis.

2.4 Conditioning Chronos Advance CRT container

1. Remove the six orange 'Cool Phase' PCM panels from the CRT container and place them un-stacked at a temperature of approximately 20°C for at least 24 hours.
2. Reassemble the container placing the bottom and side PCM panels in place, ensuring that the side with the writing is facing towards outwards (as shown in the diagram below).
3. Place samples in the silver central payload box and carefully place in the centre of the CRT container.
4. Load the final PCM panel on top of the payload box ensuring that the side with the writing is facing outwards.
5. Place the polystyrene lid onto the system ensuring it is tightly sealed.
6. Close the outer carton.

Please consult Chronos Advance Pack-Out Instructions for a video detailing the assembly instructions: (https://www.youtube.com/watch?v=dl0pRMz4CAQ)

3 Statements

3.1 Kit storage and stability

This kit is stable at 4°C for 6 months. Bring components up to room temperature before use.

Atelerix does not recommend using the kit after the expiry date stated on the packaging.

3.2 Trademarks

WellReady™ is a trademark of Atelerix Ltd.

3.3 Cellular material

Cellular monolayers can be used. Please ensure that biological material is free of fungal and bacteriological contamination before proceeding.

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